PREPARATION OF FOLLICLE STIMULATING HORMONE FROM SHEEP PITUITARY GLANDS*

C. Hashimoto, W. H. McShan and R. K. Meyer

Department of Zoology, University of Wisconsin
Madison, Wisconsin

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The preparation of highly purified follicle stimulating hormone (FSH) of sheep pituitary glands has been reported by several workers (Ellis, 1958; Duraiswami et al., 1964; Papkoff et al., 1964) but unequivocal criteria for the homogeneity of these preparations have not been achieved. In this communication, a method is reported for the isolation of follicle stimulating hormone from sheep pituitary glands. The preparation obtained by this method possessed high FSH activity and was homogeneous as indicated by physicochemical studies.

EXPERIMENTAL

Step 1. The fresh glands (1 Kg) were ground finely and extracted by a modification (Duraiswami et al., 1964) of the method of Koenig and King (1950). The hormone was precipitated by the gradual addition of acetone to the extract at -18°C, recovered and dried by lyophilization. Step 2. Subsequent purification was carried out at 4°C. The crude extract was fractionated by gel filtration on a column of Sephadex G-100 equilibrated with 0.05 M sodium chloride. The fraction containing

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FSH was rechromatographed on a column of Sephadex G-100 equilibrated with 0.004 M sodium chloride. Step 3. The separation of FSH from other proteins was performed by ion-exchange chromatography on a column of DEAE-Sephadex. The adsorbent was equilibrated with 0.01 M sodium phosphate (pH 7.0) and eluted stepwise with 0.01, 0.02, 0.05, 0.075, 0.1 M sodium phosphate buffer and 0.1 M sodium phosphate buffer containing 0.25 M sodium chloride. Step 4. The FSH fraction obtained was desalted on a column of Sephadex G-100 equilibrated with 0.004 M sodium chloride, and was further purified by gel filtration on a column of Sephadex G-100 equilibrated with 0.025 M acetate buffer at pH 5.2.

The biological activity of each fraction was determined routinely by the increase in the weight of the ovaries of intact rats (Duraiswami et al., 1964). The FSH preparations were assayed by the method of Steelman and Pohley (1953). The ovarian ascorbic acid depletion (OAAD) method of Parlow (1961) was used to determine the luteinizing hormone (LH) activity of the purified FSH. Standard FSH (NIH-FSH-S1)* and LH (NIH-LH-S1)* preparations were used as references for the bioassays.

The protein content of each fraction was determined by absorption at 280 mμ and by the biuret method (Gornall et al., 1949). Disc electrophoresis on polyacrylamide gel was performed at 4°C according to the procedure of Davis and Ornstein (1964). Ultracentrifugal analyses were carried out in a Spinco Model E ultracentrifuge. Preliminary determination of the molecular

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weight of the FSH was done by the gel filtration method of Whitaker (1963) using a column of Sephadex G-100.

RESULTS AND DISCUSSION

The purification procedure and results of a typical preparation of FSH from the crude extract are summarized in Table I. A three-fold purification of the crude extract was effected by two consecutive gel filtrations on Sephadex G-100 (Step 2). Further purification of the FSH was achieved by chromatography on DEAE-Sephadex using stepwise gradient elution with phosphate buffer. FSH of high specific activity was present in the fraction eluted with 0.10 M phosphate buffer at pH 7.0 (Step

TABLE I

Purification of FSH from Sheep Pituitary Glands

Procedure	Total Protein	F S H Activity	
	gm/Kg fresh tissue	units*/mg protein	units*/Kg fresh tissue
Step 1	4.70	0.77	3620
Step 2			
0.05 M NaCl	1.89	1.8	3400
0.004 M NaCl			
Frac. 1	1.01	2.3	2320
Frac. 2	0.63	1.5	945
Step 3**			
0.075 M phosphate	0.137	4.8	657
0.10 M " "	0.018	47	846
0.10 M " " + 0.25 M NaCl	0.381	2.2	838
Step 4***			
0.004 M NaCl	0.013	51	663
0.025 M acetate			
Frac. 1	0.0095	36	342
Frac. 2 (FSH)	0.0035	79	276

* One unit is equivalent to the FSH activity of 1 mg of NIH-FSH-S1 standard when intact rats were used for the assays (Duraiswami et al., 1964).
** Fraction 1 of Step 2 was used for the purification in Step 3.
***The fraction eluted with 0.10 M phosphate buffer was used for the purification in Step 4.

3). A small amount of LH contamination was separated from the FSH fraction by gel filtration on Sephadex G-100 (Step 4).

The biological activity of the FSH preparation obtained from Step 4 was approximately 100 times that of the crude extract. According to the results of the Steelman-Pohley assay (1953), the activity of the FSH (Step 4, fraction 2, Table I) was approximately 44 times that of the NIH-FSH-S1 standard. The results of the OAAD assays of the FSH preparation showed LH activity 0.06 times that of NIH-LH-S1 standard.

The homogeneity of the FSH preparation was studied by means of disc electrophoresis and ultracentrifugation. One main

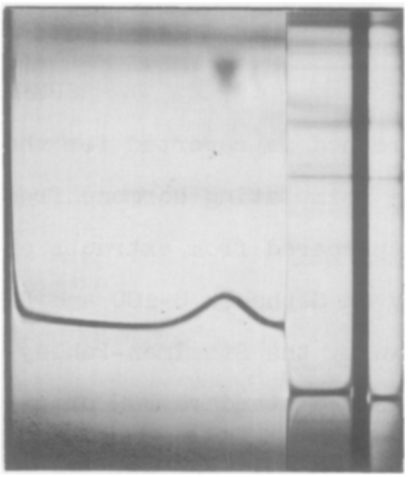

Figure 1 Figure 2

Fig. 1. Acrylamide gel electrophoresis of FSH with pH 8.6 Tris-glycine buffer for 1.5 hours at 3 mA.

Fig. 2. Ultracentrifuge pattern of FSH in 0.02 M acetate-0.1M NaCl buffer, pH 5.0, after 60 minutes at 59,780 rpm. Bar angle 60°. Protein concentration 1.47 mg per ml. Sedimentation from right to left.

zone (Rf of 0.60 - 0.63) was shown by disc electrophoresis (Figure 1). The sedimentation velocity measurement indicated the presence of a symmetrical boundary having the sedimentation constant $S_{20,W}$ of 2.6S (Figure 2). Preliminary studies by ultra centrifugal analyses and by gel filtration suggest that the molecular weight of sheep FSH is close to 33,000.

Although the results from the physicochemical analyses indicate that the FSH preparation is homogeneous, it is of interest that it showed detectable LH activity. It is not possible at this time to state whether this small amount of LH activity is due to contamination or to the intrinsic property of the FSH molecule.

SUMMARY

A method is reported for the preparation of highly purified follicle stimulating hormone from sheep pituitary glands. The FSH was prepared from extracts of the fresh glands by chromatography on Sephadex G-100 and DEAE-Sephadex. The FSH obtained was shown by the Steelman-Pohley assay to be about 44 times as active as the standard FSH preparation (NIH-FSH-S1). Physicochemical studies indicate that the FSH preparation is essentially homogeneous and the molecular weight is approximately 33,000.

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